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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	**PAPER NUMBER**
304	20

DATE MAILED:

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 8/29/94 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire three (3) month(s), 45 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 1-17, 18, 19, 20, 21-84, 85-94 are pending in the application.

   Of the above, claims 1-16, 19, 21-84 are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 17, 18, 20, 85-94 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).

12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.

13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

This application should be reviewed for errors.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-94 are pending in this application. However, claims 1-16, 19, 21-84 have been withdrawn from consideration in the parent application, application serial no. 07/726,812. The restriction as set forth in the parent application remains applicable in the instant application. Prosecution is being continued on the invention elected and prosecuted by the applicant in the parent application. Therefore, claims 17, 18, 20 and 85-94 are active and examined in this Office Action.

The two declarations of Dr. Reynolds are acknowledged, have been considered and are addressed, below.

The declaration of Dr. Weiss is acknowledged, has been considered and is addressed, below.

The provisional rejection of claims 17, 18, 20 and 85-94 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 25-35 of copending application serial no. 07/961,813 is withdrawn in view of the abandonment of the application.

The provisional rejection of claims 17, 18, 20 and 85-94 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13 of copending application serial no. 07/967,622 is withdrawn in view of the abandonment of the application.

The provisional rejection of claims 17, 18, 20 and 85-94 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of copending application serial no. 08/010,829 is withdrawn in view of the abandonment of the application.

35 U.S.C. § 101 reads as follows:

> "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

Claims 17, 18, 20 and 85-94 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of copending application Serial No. 08/376,062; claims 1-17 of copending application Serial No. 08/359,945; claims 1-10 of copending application Serial No. 08/338,730. Although the conflicting claims are not identical, they are not patentably distinct from each other because the subject matter overlaps. The claims of the applications are drawn to methods of proliferating neural stem cells in culture.

This is a *provisional* obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

The obviousness-type double patenting rejection is a judicially established doctrine based upon public policy and is primarily intended to prevent prolongation of the patent term by prohibiting claims in a second patent not patentably distinct from claims in a first patent. *In re Vogel*, 164 USPQ 619 (CCPA 1970). A timely filed terminal disclaimer in compliance with 37 C.F.R. § 1.321(b) would overcome an actual or provisional rejection on this ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 C.F.R. § 1.78(d).

The rejection of claims 17, 18, 20 and 85-94 under 35 U.S.C. 112, first paragraph, is as follows: The rejection of claims 17, 18, 20 and 85 is maintained; the rejections of claims 88, 92 and 93 are withdrawn. Applicant's arguments, filed August 29, 1994, have been considered but not found to be persuasive. Applicants have requested clarification of the statement in the previous Office Action regarding the fact "that claim 17 does not claim adult tissue and the combination of references renders obvious

the claimed invention". The examiner made the statement in response to applicant's arguments (see page 5 of the previous Office Action) and it is not apparent to the examiner what is unclear about the statement.

Applicants have argued that the data set forth in the Rule 132 Declaration (Reynolds) show that neural tissue obtained from adult mice gives rise to multipotent stem cells which can be proliferated in vitro. However, the declaration states that the methods disclosed within the declaration and used to achieve the results therein are not the same as those methods used in the specification. See paragraph 3, wherein it states:

"The article describes the isolation and proliferation of adult neural stem cells using *substantially* (emphasis added) the same techniques described in the examples of the specification".

The declaration fails to specifically disclose the differences between the technique in the specification and the technique in the reference and the differences apparent to the examiner, such as the use of bFGF in the reference technique would represent a significant difference rendering the reference methods "not substantially the same as" those of the specification. The reference apparently uses bFGF which is not used in the methods in the specification to achieve the demonstrated results. In view of the myriad actions of hormones on cell functions, one of skill would assume the addition of another hormone would give different results than experiments run without the hormone. Therefore, the declaration is insufficient to overcome the rejection since the declaration is not commensurate in scope with the teachings of the specification.

Applicants have argued that the data set forth in paragraphs 7-9 of the declaration and the photographs show that neural tissue obtained from an adult human gives rise to multipotent

stem cells which can be proliferated in vitro. However, as stated above, the declaration discloses that the method used to give the stated results is not the same as that used in the specification. The specification fails to disclose use of bFGF (page 41 of the specification) and therefore the declaration encompasses methods not taught in the specification to achieve the stated results. The declaration is not commensurate with the teachings of the specification. Therefore, the claims must be limited to non-adult tissue.

Applicants have argued that paragraph 10 of the declaration provides evidence that multipotent stem cells can be isolated from mammalian neural crest tissue and proliferated in vitro in response to treatment with amphiregulin. However, paragraph 10 discloses use of amphiregulin in the culture medium but fails to disclose the results of the control condition, which is no amphiregulin added. There is no evidence presented that the cells proliferated in response to amphiregulin, lacking adequate controls. The rejection of claim 85 is maintained.

The rejection of claim 88 regarding the lack of evidence to show that stem cells may be proliferated in vitro without any limit is withdrawn.

The rejection of claim 92, drawn to adult or juvenile tissue is withdrawn.

The rejection of claim 93 is withdrawn in view of the amendments to the claim.

The rejection of claim 93 under 35 U.S.C. 112, second paragraph, is withdrawn in view of the amendments to the claim.

The rejection of claim 87 under 35 U.S.C. 112, second paragraph, is withdrawn in view of the amendments to the claim.

The rejection of claim 17 under 35 U.S.C. 102(a) as being anticipated by Cattaneo is withdrawn in view of the amendments to the claims. Applicant's arguments are therefore moot.

The rejection of claims 17 and 85-90 under 35 U.S.C. 102(a) as being anticipated by Anchan (Neuron) is withdrawn in view of the amendments to the claims. Applicant's arguments are therefore moot.

The rejection of claims 17, 89, 91 and 93 under 35 U.S.C. 102(b) as being anticipated by Temple is maintained. Temple discloses a method for the in vitro proliferation of a multipotent neural stem cell since Temple discloses in vitro proliferation of blast cells isolated from embryonic rat forebrain, mechanical dissociation of the cells into a single cell suspension, exposing the cells to a culture medium containing at least one growth factor which caused the cells to proliferate. Temple discloses (page 472, figure 1 legend) that 55% of the mixed clones contain GFAP-positive astrocytes as well as neurons. It is known in the art that astrocytes are derived from glial cells. Therefore, Temple discloses obtaining clones derived from single cells wherein the single cell as proliferated and discloses that the single cell is capable of producing progeny which are capable of differentiating into neurons and glial cells. Regarding step (b), Temple exposed the cells to culture medium conditioned by a primary bulk culture of cells and conditioned medium is known to add growth factors or soluble cellular products necessary for growth to the medium. Therefore, Temple discloses exposing the cells to a culture medium containing at least one growth factor. Regarding step (c), Temple discloses (page 472, figure 1 legend) that clones were fed by replacing medium with fresh culture medium every 3-5 days, and therefore disclose passaging the progeny to a second culture medium containing at least one growth factor. Regarding claims 89, 91 and 93, Temple discloses that the progeny are in suspension. Figure 1 is interpreted to be that of clonally

derived neurospheres. Therefore the reference anticipates the claims.

Applicant's arguments, filed August 29, 1994, have been considered but not found to be persuasive. Applicants have argued that Temple does not describe a method whereby proliferated cells are passaged to induce further proliferation. However, contrary to such arguments, the specification fails to provide a definition of "passage" and since Temple does disclose changing of the medium, Temple has been considered to teach passsage of the cells. Note that "passage" as it is known in the art usually encompasses trypsinization of the cells to remove them from the surface of the dish or flask, dissociation of the clumps, followed by centrifugation and then resuspension of the cells in new medium in the culture flask or dish. Note that example 4 does not disclose the process of trypsinization, centrifugation, and resuspension. The term "passage" has no support in the specification. See rejection under 35 U.S.C. 112, below.

Applicants have argued that Temple does not provide evidence that her culture techniques caused proliferation of a multipotent stem cell and that the observation that a few rare cells in the culture "may be multipotential stem cells" was made simply because the cells did not stain for neurofilament or GFAP and therefore were unlikely to be differentiated neurons or astrocytes. However, contrary to such arguments, Temple discloses that dividing cells are defined as blast cells and that 58% of the blast cells divide only once. Temple is seen to have the claimed cell population,lacking evidence to the contrary. It is well settled that when a claimed product appears substantially identical to one disclosed in the prior art, the burden is on the applicant to prove that the product of the prior art does not necessarily or inherently possess characteristics of the claimed product. Although Temple did not directly assay for the presence

of nestin, the fact that the cells were capable of giving rise to glial and neuronal progeny indicates the presence of the "stem cell". Further indirect evidence supports the examiner's position. Temple discloses that the cells divided once; the prior art submitted by applicants drawn to stem cell characteristics clearly indicates that stem cells do not divide on a regular basis. See Potten, page 1002, column 2, wherein Potten discloses that stem cells do not have express their capabilities, though they posses the capabilities, and that it may be possible for a stem cell to cease proliferation, i.e, become quiescent. The stem cells of Temple fit this criteria. Potten further discloses that of the criteria listed, some terms outlined in the stem cell definition have stronger weight than others. Potten discloses that the characteristic of being undifferentiated is a fairly specific criteria but is weakest when used in its morphological sense that it has no physical features commonly attributed to specialized chemical or physical functions. Potten further discloses the various meanings of "self-maintenance" with regard to the ability of stem cells to reproduce themselves. The blast cells of Temple divided once, are therefore are considered to have reproduced themselves, and thus proliferated. Note that the claims do not claim that the progeny be stem cells and the examiner has interpreted the word "progeny" as a daughter cell resulting from a cell division. A "progeny" of a stem cell may be the differentiated daughter cells. The specification lacks a definition of "progeny".

Applicants have argued that their neurospheres would have several hundred cells after only 6-7 days in culture. However, the claims do not claim any particular number of cells in the neurospheres.

Applicants have argued that Temple does not disclose culture in suspension and points out that the cells of Temple were

culture in poly-L-lysine coated wells as disclosed in figure 1, legend. However, the figure legend discloses that the

"Each well had a pre-established culture of striatal cells growing on its walls but not on its base. These wall cultures were established by inoculating each poly-L-lysine coated well with 10 ul of a suspension of striated cells in ...medium... while holding the Terasaki plate in an inverted position. The plates were incubated in this position for 6 days and then righted...".

Therefore, as best can be determined by the examiner, the poly-L-lysine was only on the walls of the well, not on the based. Therefore, Temple disclosed a suspension culture. Regarding the appearance of the cells, the examiner has based the interpretation of the figures on the description of figure 1 a-e. It is well known that when cells divide in suspension, they divide to form three dimensional clumps and in order to determine that there were 12 cells, the cells must have been in an aggregate, therefore in suspension. Clearly a better picture may resolve the issue.

The rejection of claims 17, 18, 85-88, 90 and 93 under 35 U.S.C. 102(a) or (b) as being anticipated by Reynolds et al. (Abstract 474.2)is withdrawn. The declarations submitted by Drs. Reynolds and Weiss are sufficient to overcome the rejection.

The rejection of claims 17, 18, 85-88, 90 and 93 under 35 U.S.C. 102(f) Reynolds et al. (Abstract 474.2)is withdrawn. The declarations submitted by Drs. Reynolds and Weiss are sufficient to overcome the rejection.

The rejection of claims 17, 18, 85-88 and 90 under 35 U.S.C. 102(f) Reynolds et al. (J. of Neuroscience, volume 12(11), 1992)is withdrawn. The declarations submitted by Drs. Reynolds and Weiss are sufficient to overcome the rejection.

The rejection of claims 91 and 92 under 35 U.S.C. 103 as being unpatentable over Anchan (Neuron) as applied to claims 17,

85-90 above, and further in view of Reh, is withdrawn in view of the amendments to the claims. Applicant's arguments are moot.

The rejection of claims 85-90 under 35 U.S.C. 103 as being unpatentable over Cattaneo as applied to claim 17 above and further in view of Anchan (Neuron) is withdrawn in view of the amendments to the claims. Applicant's arguments are moot.

The rejection of claims 17, 85, 86, 89 under 35 U.S.C. 102(b) as being anticipated by Anchan (Abstract 308, JCB, volume 109, 1989) is withdrawn in view of the amendments to the claims. Applicant's arguments are moot.

The rejection of claims 18, 20, 87, 90-93 under 35 U.S.C. 103 as being unpatentable over Anchan (abstract 308) as applied to claims 17, 85, 86, 88 and 89 above and further in view of Anchan (Neuron) is withdrawn in view of the amendments to the claims. Applicant's arguments are moot.

The following are new grounds of rejection.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make the invention as claimed, i.e., failing to provide an enabling disclosure. The specification discloses EGF induced development of a cluster of undifferentiated cells from a single cell (pages 15-16)and discloses culture of the cells for 10 cell DIV. The specification further discloses testing of the cells for nestin expression after 10 DIV. The specification fails to disclose that the nestin+ cells were then passaged to a second culture medium

to proliferate said progeny. The specification fails to disclose that nestin+ cells can be passaged multiple times to produce progeny wherein the progeny are nestin+ cells and not differentiated. Therefore, the specification fails to enable one of skill to practice the invention as claimed. In view of the complexities of cell cycle of stem cells and in view of the problems associated with measuring stem cells (see for example, Potten), undue experimentation would be required by one of skill to practice the invention as claimed since there is no evidence in the specification that a second round of passage would result in further proliferation of the stem cell. Note that the examiner has defined the word "progeny" as stem cell daughters which are themselves stem cells.

Claims 17, 18, 20 and 85-94 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 17 18, 20 and 85-94 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Regarding independent claim 17, the word "progeny" is vague and unclear since it is not evident if progeny refers to progenitor cells or to other stem cells. Further, the preamble claims a method for the in vitro proliferation of a multipotent stem cell but the body of the claim claims progeny.

Regarding independent claim 17, the word "passaging" is vague and unclear since applicants apparently mean transfer. Further, the word "passaging" lacks literal support in the specification and what applicants intend "passaging" may be different from the art-recognized term.

Claims 17, 18, 20, and 85-94 are rejected under 35 U.S.C. § 103 as being unpatentable over Anchan taken with Boss. Anchan

discloses a method for the in vitro proliferation of neural stem cells comprising dissociation of rat neural tissue containing at least one multipotent stem cell capable of producing progeny capable of differentiating into neurons and glial cells. See page 923, column 2, "Results". Anchan discloses dissociating the retina to obtain a single cell suspension and culturing the cells in the presence of either EGF or TGF-alpha. Anchan further discloses plating the cells on a substrate of poly-L-lysine. Anchan differs from the claims in that the reference fails to disclose culture in a defined, serum free medium or passaging the cells as singlets or as aggregates. However, the secondary reference, Boss, cures the deficiency. Boss discloses (column 3, "Summary") depending on the culture conditions and period, the progenitor cells differentiate either in vitro or in vivo; that the culture can be progenitor cells or aggregates of progenitor cells in a culture medium, or single or aggregated neuron progenitors cells on or dispersed in a substrate matrix; that most preferably the cultures are suspension cultures in which the progenitor cells grow as aggregates. Boss further discloses that the method comprises culturing the neuron progenitor cells in an initial culture medium which selects for a novel cell culture containing neuron progenitor cells (adaptive period) and growing the cells for a period of time (growth period) in a second medium. Boss discloses that the progenitor cells can be induced to differentiate in vitro by addition of a differentiation agent. Boss discloses that subculture is synonymous with "passage". Boss discloses that the obtained tissue is from human or porcine sources. Boss discloses the preparation of monolayer and suspension cultures from the dissociated cells (column 5); that the aggregate size is controlled by the culture conditions. Boss discloses that his cell population contains neural stem cells since (column 6) "... cells migrating from these (3-D) structures

and form typical two dimensional monolayers in which differentiating neurons and glial cells can be observed. Boss discloses that (column 7) the cultures are initially grown in a first culture medium which promotes the survival of neuron progenitor cells which are capable of proliferating in a serum free-defined medium and that the initial culture medium can be supplemented with hormone and growth factors. Boss discloses passaging the cells (column 12) followed by plating on a fixed substrate, poly-L-ornithine (claim 90) or alternatively reculturing in aggregates (claim 91). Regarding claim 88, Boss discloses (column 12) that on day 5 after seeding, the medium should be exchanged every 3-4 days. Boss therefore discloses repeating step (c) at least one additional time.

Regarding claim 92, Boss discloses use of juvenile tissue.

Regarding claim 94, it would have been obvious to one of ordinary skill to repeat the process in order to expand the cell culture to different flasks, lacking evidence to the contrary. Maintenance and expansion of cell cultures is old and well known in the art as the concept of transferring cells to fresh medium.

It would have been obvious to one of ordinary skill to modify the cell culture method of Anchan by passaging the cells in a defined culture medium, lacking serum, on a fixed substrate as suggested by Boss in order to obtain a method for the in vitro proliferation of multipotent neural stem cells capable of producing progeny capable of differentiating into neurons and glia. One of ordinary skill would have had a reasonable expectation of success in modifying the method of Anchan in view of the teachings of Boss that monolayers produce cells capable of differentiating into neurons and glia (column 6, lines 9-12). Apparently, the production of neurons and glia as well as the self-renewal of progenitor cells from neural precursor cells is independent of culture method, lacking evidence to the contrary.

Accordingly, the modification of the method of Anchan by subculturing the cells both in suspension and on a fixed matrix as suggested by Boss in order to obtain a method for the in vitro proliferation of a multipotent neural stem cell was within the ordinary skill in the art at the time the claimed invention was made. From the teachings of the references, it is apparent that one of ordinary skill would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole is prima facie obvious, as evidenced by the references, especially in the absence of evidence to the contrary.

No claim is allowed.

Papers related to this application may be submitted to Group 1800 by facsimile transmission. Papers should be faxed to Group 1800 via the PTO FAX center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (30 November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Suzanne Ziska, Ph.D., whose telephone number is (703)308-1217. In the event the examiner is not available, the examiner's supervisor, Ms. Jacqueline Stone, may be contacted at phone number (703) 308-3153.

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